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BARD

FINAL REPORT

PROJECT NO. IS-2760-96

Genetic Transformation of Flowering Bulb Crops for Virus Resistance

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BARD Final Scientific Report (Cover Pages)

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Project Title: Genetic transformation of flowering bulb crops for virus resistance

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*Passed away during the course of the project.

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Easter lily, Gladiolus, cucumber mosaic virus, bean yellow mosaic virus, aphid transmission, monoclonal antibody genes

Abbreviations commonly used in the report, in alphabetical order:

BYMV (bean yellow mosaic virus), CMV (cucumber mosaic virus)

MAB (monoclonal antibody)

Publication & Patent Summary (following page)

Summary of Cooperation (following page)

Budget: IS: \$149,190

US: \$150,810

Total: \$300,000

Abed Gera

Signature
Principal Investigator

J. Spied

Signature
Authorizing Official, Principal Institution

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	1		1	2
Submitted, in review, in preparation		1		1
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts	1	1	2	4
Not refereed (proceedings, reports, etc.)	1	3	2	6

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Dr. Slava Andrianov (Social security number -- 069-84-2735)- US
Dr. Alex Lipsky - IL

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		1		1
Longer Visits (Sabbaticals)		1		1

Description of Cooperation:

Dr. Abed Gera visited the USDA in 2000 while attending a conference in Annapolis, MD, and he saw the progress made on the collaborative projects. Dr. Avner Cohen is on his sabbatical at the USDA for one year beginning 8/2000, and he spends a portion of his time working on the BARD project.

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Abstract

Objectives. The major aim of the proposed research was to establish an efficient and reproducible genetic transformation system for Easter lily and gladiolus using either biolistics or *Agrobacterium*. Transgenic plants containing pathogen-derived genes for virus resistance were to be developed and then tested for virus resistance. The proposal was originally aimed at studying cucumber mosaic virus (CMV) resistance in plants, but studies later included bean yellow mosaic virus (BYMV). Monoclonal antibodies were to be tested to determine their effectiveness in interfering with virus infection and vector (aphid) transmission. Those antibodies that effectively interfered with virus infection and transmission were to be cloned as single chain fragments and used for developing transgenic plants with the potential to resist virus infection.

Background to the topic. Many flower crops, as lily and gladiolus are propagated vegetatively through bulbs and corms, resulting in virus transmission to the next planting generation. Molecular genetics offers the opportunity of conferring transgene-mediated disease resistance to flower crops that cannot be achieved through classical breeding. CMV infects numerous plant species worldwide including both lilies and gladioli.

Major conclusions, solutions and achievements. Results from these collaborative studies have demonstrated the potential for future development of transgenic floral bulb crops for virus resistance.

In Israel, an efficient and reproducible genetic transformation system for Easter lily using biolistics was developed. Transient as well as solid expression of GUS reporter gene was demonstrated. Putative transgenic lily plantlets containing the disabled CMV replicase transgene have been developed. The *in vitro* ability of monoclonal antibodies (mAbs) against CMV to neutralize virus infectivity and block virus transmission by *M. persicae* were demonstrated.

In the US, transgenic Gladiolus plants containing either the BYMV coat protein or antisense coat protein genes have been developed and some lines were found to be virus resistant. Long-term expression of the GUS reporter gene demonstrated that transgene silencing did not occur after three seasons of dormancy in the 28 transgenic Gladiolus plants tested. Selected monoclonal antibody lines have been isolated, cloned as single chain fragments and are being used in developing transgenic plants with CMV resistance.

Ornamental crops are multi-million dollar industries in both Israel and the US. The increasing economic value of these floral crops and the increasing ban numerous pesticides makes it more important than ever that alternatives to chemical control of pathogens be studied to determine their possible role in the future.

The cooperation resulted in the objectives being promoted at national and international meetings. The cooperation also enabled the technology transfer between the two labs, as well as access to instrumentation and specialization particular to the two labs.

Achievements

These results have demonstrated the potential for future development of transgenic floral bulb crops for virus resistance. Many experiments were conducted to repeat the transformation of *Lilium longiflorum* cv. Osnat, as already reported in the literature. Despite the bombardment of thousands of leaf and callus explants, and the recovery of many putative transgenic plants we were unable to confirm the presence of a whole transgenic plant. We were unable to find a transgenic chimeric plant, although we have had putative transgenic plants containing the disabled CMV replicase transgene, that did survive on the Basta-containing media. Finally, we have been able to obtain apparently solid transformants of cv. Osnat following bombardment with a plasmid based on pCAMBIA, constructed by Dr. R. Barg (ARO Volcani Center, Bet Dagan, Israel). Now there are apparently many transformants expressing the GUS gene, some of which appear solid blue after GUS staining. However, in addition some of the plants appear chimeric with GUS expression in patches. The plants are currently small, and will be tested further after growth, including Southern blotting.

Seven affinity-purified monoclonal antibodies (mAbs) produced in Beltsville¹ against (CMV) subgroup I and II, were screened for their ability to neutralize virus infectivity *in vitro*. Infectivity was inhibited almost completely when virus preparations were mixed *in vitro* with the homologous mAb prior to inoculation. The most efficient mAbs were 4H10B12 and 6D11D12 for subgroups I and II, respectively. The ability of the mAbs to block virus transmission by *Myzus persicae* was also tested; both, pre- and post-acquisition treatments of aphids with mAbs significantly decreased virus transmission. Treatment with mAb 6D11D12 before or after feeding on purified virus preparations prevented CMV transmission almost completely.

Transgenic Gladiolus plants containing either the BYMV coat protein or antisense coat protein genes have been developed and some lines were found to be virus resistant. Antiviral testing of the transgenic Gladiolus plants containing genes against BYMV resistance could not have been achieved without the expertise of the Volcani group who did the challenging. The methods developed by Volcani researchers for challenging the transgenic Gladiolus BYMV will be shared with the USDA group and applied to challenging the population of transgenic Gladiolus plants containing antiviral genes against CMV. Previously virologists at the USDA have attempted challenging of transgenic Gladiolus plants with BYMV, but they were not successful.

Five vectors for developing transgenic plants resistant to CMV by biolistics have been constructed at USDA and shared with the Volcani. These include the CMV coat protein subgroup 1 gene, CMV coat protein subgroup 2 gene, and defective CMV replicase gene. USDA has cloned the MAb gene to CMV and is currently testing this construct in *Nicotiana benthamiana*. A vector was constructed for expression of CMV replicase in plants that utilized translational fusion with *uidA* and self-cleavage via a potyvirus proteinase. This construct allows detection of CMV replicase expression by monitoring GUS expression which should be much easier than isolating the replicase protein or its RNA for detection of replicase expression. All constructs will be available

to Volcani.

Transgenic *Gladiolus* plants containing the reporter gene *uidA* under four different promoters (CaMV 35S, mannopine synthase, *rolD*, and ubiquitin from *Arabidopsis*) have been developed. Long-term GUS expression was demonstrated in the 23 transgenic plants after three seasons of dormancy. *Gladiolus* is propagated by corms that undergo dormancy each year, and at least three seasons of growth are required before the plants from tissue culture produce flowers. Successful genetic engineering of the flowering bulb crops will require long-term expression of transgenes, and this study demonstrated that transgene silencing will not be a problem in genetic engineering of bulb crops.

Tissue-specific expression of the *uidA* gene under the four promoters (CaMV 35S, mannopine synthase, *rolD*, and ubiquitin) tested was analyzed. Development of transgenic plants against a pathogen requires that the promoter direct transgene expression in the particular plant tissues that will be invaded by the pathogen. In *Gladiolus* the highest levels of GUS expression were found in the callus, shoots, and roots of plant cells containing the *uidA* gene under the CaMV 35S promoter. It was found critical to analyze levels of stable expression in transformed plants because the levels were different from those found following transient expression. Twelve of 15 plants contained 1-2 copies of the transgene, and there was no effect of copy number on levels of GUS expression. Twelve of 23 plants analyzed did not show rearrangements of the transgene, and there was no effect of rearrangements on levels of GUS expression.

Expression of a transgene can be affected by environmental conditions that the plant is grown under. There was no significant difference in GUS expression for transgenic *Gladiolus* plants grown *in vitro* as compared to plants grown in the greenhouse indicating that transgene expression can be detected *in vitro*.

Agricultural and/or economic impacts of the research findings:

Lilium and *gladiolus* species are significant floriculture commodity among the major bulb crops in the commercial market. This industry is valued around millions of US dollars. CMV causes a serious disease of both lily and *gladiolus*. Partly through the efforts of this project, the transgenic plants, if proved to be efficient against CMV, then this strategy could be applied to other sensitive geophytes.

Details of cooperation:

The principal investigators in Israel and the US have maintained almost daily communication via e-mail and fax. Constructs containing sense and antisense orientations of CMV CP, vectors, primers and antibodies have been shared. Protocols for transformation of lily, methods for challenge inoculation of transgenic plants and aphid transmission of CMV were exchanged. Dr. Abed Gera visited the USDA in 2000 while attending a conference in Annapolis, MD, and he saw the progress made on the collaborative projects.

Publication resulting from the present BARD project:

Gera, A. Hsu, H.T. Cohen, J. Watad, A. Beckelman E. and Hsu, Y.H. (2000). Effect of cucumber mosaic virus monoclonal antibodies on virus infectivity and transmission by *myzus persicae* J. of Plant Phytopathol. 82, 3-7.

Kamo, K. (2000). Expression of the bar and uidA genes by Gladiolus following three seasons of dormancy. Proceedings of the IV In Vitro Culture and Horticultural Breeding Symposium. Acta Hort.(In press)

Lipsky, A., Cohen, A., Gaba, V., Kamo, K., Gera, A., and Watad, A. (2001). Transformation of *Lilium longiflorum* plants for cucumber mosaic virus (CMV) resistance by particle bombardment. Proceedings of the 10th International Symposium on Virus Diseases of Ornamental Plants. Acta Hort.(In press).

Watad A., Yun, D. J. Matsumoto, T., Niu, X., Wu, Y., Kononowicz, A. K. Bressan, R.A. and Hasegawa, P. M. 1998. Microprojectile bombardment-mediated transformation of *Lilium longiflorum*. Plant Cell Report 17, 262-267.